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ORIGINAL ARTICLE

Effects of honey, glucose, and fructose on the enamel demineralization depth

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Abstract *Background/purpose:* Caries prevention is an important strategy in many countries. Honey has antibacterial activity against cariogenic bacteria and therefore may have less caries activity than other sugars. This study was conducted to compare the cariogenic effect of honey with those of glucose and fructose.
Materials and methods: In this *in vitro* study, 36 extracted caries-free human premolars were collected and prepared following a multistage laboratory process. Then, the teeth were randomly divided into three groups. Each group was put into separate tubes containing different solutions of honey, fructose, and glucose in a brain-heart infusion broth environment. About 1.5×10^8 cells of *Streptococcus mutans* (equal to 0.5 McFarland units) were added to each tube. Every other day, 2 ml of the solution were replaced by 2 ml of a previously prepared solution for 21 days. Teeth were sectioned buccolingually using a diamond-saw microtome. The demineralization depth of each section was measured at three points, and the average of three representative measurements was considered the lesion depth.
Results: Mean $\pm$ SD demineralization depths related to honey, glucose, and fructose were 160.1 ± 59.82 μm, 245.98 ± 96.13 μm, and 195.98 ± 47.53 μm, respectively. Differences among the three means were statistically significant.
Conclusion: The results of this study demonstrated that honey had less caries activity than the other sugars. However, further evidence is required to detect the active components and

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mechanisms by which it reduces demineralization and to demonstrate whether this food has any clinical application for preventing and reducing dental caries.

Introduction

Dental caries are one of the most common, communicable, and intractable infectious diseases in humans.[1] They remain a persistent and important oral health problem in the world particularly in developing countries. They affect 90% of young adults in the USA.[2] Almost 43% of 5-year-old children in England and 61% in Northern Ireland had dental caries in 2003.[3]

Bacterial action on fermentable dietary carbohydrates leads to the production of acids, diffusion into the teeth, demineralization, and ultimately the formation of dental caries.[4] Acidogenic bacteria, salivary dysfunction, dietary carbohydrates, the use of fluoride, oral hygiene, lifestyle, health education, and genetics are some of various factors that may affect dental caries progression.[3,5–7]

Honey has been used as a medication since ancient times.[8] Natural antioxidants and flavonoids exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, and antithrombotic activities.[9] Honey was indicated to possibly clear infections, remove malodors, reduce inflammation and pain, and cause edema and exudation to subside. In addition, honey has healing properties by stimulating angiogenesis. Honey has the potential to be useful for periodontal therapy, the prevention of infection in wounds following extraction or oral surgery, erosion of the mucosa, radiotherapy-induced stomatitis, and oral ulcers without adverse reactions.[10–13]

Many studies have reported the antibacterial activity of honey, but there are few investigations into the effect of interactions of honey with cariogenic bacteria on dental caries. The aim of this study was to investigate the cariogenic effect of honey compared to that of glucose or fructose.

Materials and methods

This study was conducted in Hamadan City, western Iran, in June 2011. Three different sugars were used: fructose, glucose (Merck, Darmstadt, Germany), and natural honey. Honey was directly obtained from local beekeepers in order to assure that no artificial preservatives or other sugars had been added.

In this *in vitro* study, 36 extracted caries-free human premolars were collected and stored in a solution of 10% formalin in order to disinfect and sterilize the extracted teeth and prevent bacterial growth, which may otherwise remain viable within the root canals of the teeth.[14–16] No developmental defects, cracks, caries, or white spots were found on the buccal enamel surface of the teeth. Pretreatment with a chemical agent such as hydrogen peroxide had not been performed on any of the teeth. After removing the remaining soft tissue with a razor blade, the teeth were cleansed and polished with nonfluoridated pumice and rubber prophylactic cups. The root apexes of each tooth were sealed with red wax, and all surfaces of the teeth except the buccal surface were covered with nail polish.

Brain-heart infusion (BHI) broth medium (Merck) was prepared and sterilized in an autoclave for 15 minutes at 121°C and 15 [pounds/use CGS units for pressure] of pressure. Each tooth was placed in a separate test tube with 3 mL BHI broth and then incubated in a 37°C incubator. After 24 hours, the cloudiness of the samples was evaluated to ensure the absence of contamination. Teeth were removed from the tube using sterilized forceps and randomly divided into three groups. Each group was put into new tubes containing 5 mL of three different solutions prepared as follows. Tube 1 included 20 g of honey plus 80 mL sterilized BHI broth media. Tube 2 included 20 g of fructose plus 20 mL distilled water that had passed through a cellulose membrane, and then 80 mL of sterilized BHI broth media was added. Tube 3 included 20 g of glucose plus 20 mL distilled water that had passed through a cellulose membrane, and then 80 mL of sterilized BHI broth media was added.

About 1.5×10^8 cells of *Streptococcus mutans* PTCC 1683 (equivalent to 0.5 McFarland units) was added to each tube. Every other day, 2 mL of the solution was replaced by 2 mL of a previously prepared solution for 21 days. The extracted solutions were cultured in (nutrient) agar (Merck) plates to ensure the absence of contamination with any bacteria other than *S. mutans*.

The teeth were sectioned buccolingually using a diamond-saw microtome (Hammarlund-Essler, Stockholm, Sweden) to obtain sections of approximately 300 μm in thickness. A final polishing was performed in order to attain the desired thickness (100 μm) for histological examination using a high-capacity grinding microtome (Seastrand/Original-Müller-Tempo, Stockholm, Sweden). Sections were then immersed in water (with a refractive index of 1.33) for evaluation under polarized light microscopy using an SZX 12 Olympus (Olympus, Tokyo, Japan) microscope. The demineralization depth of each section was measured at three points, and the average of the three representative measurements was considered the lesion depth (Fig. 1).

The mean depth of demineralization of each experimental group was compared using a *t* test. All statistical analyses were performed at a 95% significance level using Stata 11 statistical software (StataCorp, College Station, TX, USA).

Results

As shown in Table 1, the mean demineralization depth induced by honey was less than the mean demineralization depths induced by glucose ($P < 0.001$) and fructose ($P = 0.006$). In addition, the mean demineralization depth

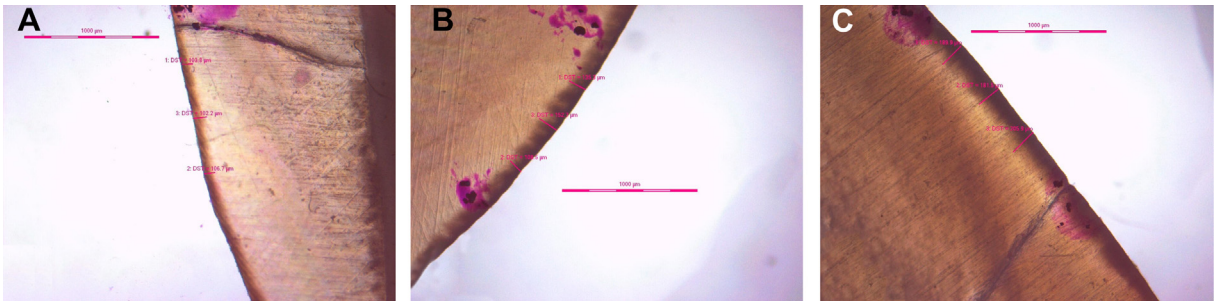

Figure 1 Histological examination of the enamel demineralization depth measured at three points under polarized light microscopy. (A) Honey-induced demineralization; (B) fructose-induced demineralization; (C) glucose-induced demineralization.

induced by glucose was less than that induced by fructose ($P = 0.007$).

Discussion

Dental caries are a common infectious and communicable disease with multiple risk factors such as genetic, environmental, diet, bacterial, and lifestyle factors that influence the initiation and progression of dental caries.[6,17,18]

Sweeteners are one of the most common causes of dental caries.[18] In addition, honey is a super-saturated, delicious, and naturally sweet nectar popular worldwide and is collected by bees from a wide variety of plants. Thus, using a sweetener with a lower chance to do harm in the diet such as honey is important.

Honey has antibacterial activity against many bacteria such as two major cariogenic bacteria *S. mutans* and *Lactobacillus*.[19,20] In addition, results of a recent study indicated that honey has less caries activity than glucose and fructose. Therefore, the antibacterial activity of honey against cariogenic bacteria and its other beneficial properties may reduce its caries activity compared to other sugars. Accordingly, honey can be used instead of other cariogenic sugars as a sweetener and as a material in toothpaste, gum, candy, chocolate, and so on.

The results of previous studies showed that fructose and glucose had lower caries production than sucrose.[21] Accordingly, it is possible that honey has less caries activity than other sugars such as fructose and sucrose. It should be remembered, however, that the lower caries activity of honey depends on many good properties of this food, which all act together.

Factors that are effective in antimicrobial activity of honey include the osmotic effect, enzymatic glucose oxidation reaction, production of hydrogen peroxide, high osmotic pressure, a low pH, an acidic environment, and the presence of phenolic acids, lysozyme, flavonoids, phytochemicals, antioxidants, beeswax, nectar, pollen, and propolis.[6,9,19,22–24]

In addition to the many beneficial effects of honey, its presence in the oral cavity seems to be very useful against dental caries and many other oral problems. One study suggested that honey contains factors that may reduce the solubility of exposed enamel in an acid buffer solution, compared to pure sucrose.[25] In addition to the solubility-reducing substances, honey contains factors that may also reduce bacterial effects on dental caries.[25] Furthermore, in spite of its high sugar content, honey was reported to have antibacterial activity in the oral cavity.[22,26]

The antibacterial activity and composition vary with the source of the honey and the way it is processed. Thus, honey should be protected from light in order to maintain its antibacterial effect.[25] In this study, we used fresh natural honey extracted from the honeycomb. However, different honeys from various sources may have different properties.

This study had a few limitations. First, the measurements were done by one observer rather than two observers, and the results might thus have been prone to a measurement bias. Second, the study was conducted *in vitro*. This may limit the generalizability of the results to clinical applications.

In conclusion, results of this study demonstrated that honey had lower caries activity than fructose and glucose. However, further evidence is required to demonstrate whether this food has any clinical application for preventing and reducing dental caries.

Table 1 Mean comparison of the demineralization of premolar teeth by three types of sugar using the *t* test.

Sugar	Number of measurements	Mean (μm)	Standard deviation	95% Confidence interval		P
Honey	36	160.01	59.82	139.77	180.25	Reference
Glucose	36	245.98	96.13	213.46	278.51	<0.001
Honey	36	160.01	59.82	139.77	180.25	Reference
Fructose	36	195.98	47.53	179.90	212.06	0.006
Fructose	36	195.98	47.53	179.90	212.06	Reference
Glucose	36	245.98	96.13	213.46	278.51	0.007

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